WGS Applications in FDA’s Foodborne Outbreak Response

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Coordinated Outbreak Response and Evaluation (CORE) Network

IFSH
May 6, 2019
Topics

• CORE Background

• Examples of WGS impact on traditional outbreak investigations

• Retrospective outbreak investigations

• Challenges & Opportunities
Role of FDA in Foodborne Outbreak Investigations

- Traceback of suspected foods to their source
- Food and environmental testing
- Communications – Public, internal, congressional
- Product and regulatory actions
- Environmental assessments of farm or production facilities
- Regulations and guidance to prevent outbreaks
Coordinated Outbreak Response and Evaluation (CORE) Network

Launched August 1, 2011, and currently includes six multidisciplinary teams

Created to coordinate FDA’s activities
  – Signal Evaluation/Surveillance
  – Response (three teams)
  – Post-Response
  – Communications

Outbreaks due to FDA-regulated food, dietary supplements, and cosmetics
Role of States in Foodborne Outbreak Investigations

• Multiple state and local agencies are involved in foodborne outbreak investigations: Health, Agriculture & Environmental Health Departments, Public Health Laboratories

• States (and localities) detect and respond to localized foodborne outbreaks and collaborate with federal agencies during national investigations

• States complete WGS of food, environmental, and clinical isolates

• Participate in PulseNet and (in some cases) Genome Trakr
WGS and The Changing Face of Foodborne Outbreaks

- Replaces PFGE as primary molecular characterization
- FDA uses WGS to identify links between FDA product or environmental isolates and clinical isolates
  - *Listeria monocytogenes*, STECs, and *Salmonella*
  - Routine and dynamic comparisons between sequences of food/environmental isolates and sequences for clinical isolates
- FDA performs WGS on all foodborne pathogen isolates from FDA samples

Use of WGS for foodborne outbreak detection and response should result in:
- Identification of more clusters, smaller case counts
- Shift in temporal boundaries of an outbreak (longer range)
- Greater clarity of relationships between isolates
- Improved targeting of resources
- Enhanced foodborne illness attribution
- More retrospective outbreak investigations

WGS is one of many pieces of the outbreak puzzle
**E. coli O121 in Flour, 2016: The Epi Signal**

- **February 2016**
  - CDC notifies CORE Signals of *E. coli* O121 cluster (PFGE based)
  - Focused questionnaire developed with emphasis on romaine, broccoli, and beef
- **March 2016**
  - No clear signal from focused questionnaire
  - CDC conducts open-ended interviews
- **April 2016**
  - 9/9 home bakers identified
  - 6/10 report consuming raw dough

**Epidemic curve indicative of vehicle with longer shelf-life**

**WGS used to:**
- Demonstrate that a 2015 beef isolate matching by PFGE was *not* highly related to clinical cluster isolates
- Refine case definition, support common source hypothesis
**E. coli O121 in Flour, 2016: Traceback and Laboratory Evidence Confirm the Vehicle**

- Restaurant 1, MD  
  Likely pack date: 11/18/2015

- Restaurant 2, VA  
  Likely pack date: 11/18/2015
  
- Restaurant 3, TX  
  Likely pack date: 1/6/2016

- Colorado, Brand A flour  
  Manufacture date: 11/14/2015
  
- California, Brand A flour  
  Manufacture date: 11/22/2015

- Washington, Brand A flour  
  Manufacture date: 11/15/2015

- Oklahoma, Brand A flour  
  Manufacture date: 11/10/2015

**Distributor A**  
VA and TX locations  
No product manipulation

**Manufacturer A**  
Flour Mill, MO  
Product: Brand A flour  
Product: Foodservice flour

**Epidemiologic Signals** point to flour

Raw dough exposure at restaurants noted.

Flour supplier and likely manufacture dates ascertained – recall initiated 5/31/16

Positive product sample confirmed by FDA

Positive product sample confirmed by FDA outside of recall bracket. Recall expanded 7/1/16
Example: Bringing PFGE Patterns Together (LM/bagged salad mix)

1509MLGX6-1WGS

wgMLST Analysis

<table>
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<tr>
<th>Id</th>
<th>State Id</th>
<th>PFGE Ascl pattern</th>
<th>PFGE Apal pattern</th>
<th>Outbreak</th>
<th>Serotype</th>
<th>Source Site</th>
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NOTE: Removed IL_134923 as part of the analysis though it is included within the 4[0-12] range of isolates

Pending WGS results on:
CA__ML5073038
NY__EN90090292604
CT__562143081

PFGE Ascl

PFGE Apal

PFGE Ascl pattern | PFGE Apal pattern
------------------|----------------------
GX6A18.0135 | GX6A12.2837
GX6A16.0135 | GX6A12.0348
GX6A16.1261 | GX6A12.0348

Three different PFGE pattern combinations in this WGS analysis.

WGS analysis by Enteric Diseases Laboratory Branch, CDC

Updated: 2016-01-15
### Example: Breaking Patterns Apart

**(Salmonella Pseudo-outbreak)**

**1601AZJPX-1 Salmonella Typhimurium**

<table>
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<th>WGS_ID</th>
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*Note: 2016K-0112 was excluded because it was different from everything else by 32,000 SNPs.*

**Settings:** 
LyveSET 1.1.4e used with reads trimmed using fastx_trimmer 5 bases from 5’ ends before mapping by SMALT. SNPs were called using Varscan at > 20x coverage, > 95% read support, and < 5 bp apart.

**Reference:** Complete genome of str. 140285 (CP001363 and CP001362) used as reference with prophage regions masked at 974189-985099, 1055358-1102365, 1281623-1335316, 1969240-1983175, 2090438-2145704, 2780087-2830859, 290589-295252, and 4341457-4451876.

**Same PFGE pattern, yet wide variation in WGS**

The methods used in the analysis of this sequence data are preliminary and remain under validation. Please email dfge@cdc.gov if you plan to use/distribute this phylogeny further.

2016-03-10

WGS analysis by Enteric Diseases Laboratory Branch, CDC
Example: Cross-Temporal Connections - *Salmonella* Anatum in Maradol Papayas 2017

Investigation begins - Papaya suspected as vehicle based on case exposures but traceback did not converge, no sampling conducted

Subsequent sampling of imported papayas in August 2017 yielded a positive sample matching an older cluster by PFGE and WGS

Investigation ends with papaya suspected

**WGS**
- Confirmed papaya as the outbreak vehicle
- Demonstrated the contamination was an ongoing issue over multiple months
Example: Firm A could not have supplied cases

- In 2015, FDA and CDC investigated a clinical cluster of *Listeria monocytogenes* with no clear epi signal.
- During the investigation, an FDA positive sample was found at Firm A; sample collection was unrelated to the investigation.
- Distribution and manufacturing information shared by field office for review by CORE.

Firm A could not have supplied product to cases based on distribution and shelf-life of the sole product manufactured.
Example: Firm B did not supply cases

- Outbreak occurred
  - Single State
  - Common supplier of meals
  - No specific vehicle identified
- Positive product sample from Firm B
  - About two months after outbreak
  - Matched the outbreak strain
  - Recall of product occurred
- Exposure
  - Cases may be have been served the commodity
  - Uncertainty around whether commodity was actually consumed
- Traceback/Traceforward
  - Documents collected did not connect Firm B to common caterer

While Firm B may have supplied the cases based on geographic distribution of the commodity in question, records demonstrated that Firm B did not supply those specific cases
Example: Firm C likely supplied some cases

- Positive product sample from Firm C
  - Not a RTE food
  - Product ultimately recalled
- Cluster of illnesses identified
  - Investigated as a result of positive product
  - Limited number of ill persons
- Exposure
  - At least one case had exposure to specific product either from Firm C or a different firm
  - Other cases had exposure to commodity, but not Firm C
- Traceback
  - Commodity for some cases would have been from a different firm
  - Due to limited number of cases, further traceback was not practical

Based on the positive sample and limited epi data, Firm C likely supplied product to some cases, but limited traceback and additional epi showed Firm C could not have supplied all cases
Traditional versus Retrospective Outbreak Investigations

Investigation begins with a cluster of illnesses

Epidemiology identifies a suspect food vehicle

Positive sample and/or Traceback Confirms Link Between Illness and Vehicle

Public Health and Regulatory Actions Occur

Investigation begins with a possible vehicle (positive sample)

Molecular tools (PFGE, WGS) identify suspect cases of illness

Epidemiology and/or Traceback Confirms Link Between Illness and Vehicle
Traditional versus Retrospective Outbreaks in Nut Butters

Traditional Outbreak Investigations

  715 cases, 129 hospitalizations, 0 deaths
  714 cases, 166 hospitalizations, 9 deaths
- *Salmonella* Bredeney (Company C/Brand C Peanut butter, 2012):
  42 cases, 10 hospitalizations, 0 deaths

Retrospective Outbreak Investigation

- *Salmonella* Braenderup (Company D/Brand D nut butter, 2014):
  6 cases, 1 hospitalization, 0 deaths
WGS Strengthens All Lines of Evidence

Direct Impact on Laboratory Evidence

- Greater confidence in isolate relatedness
- Breaks PFGE clusters apart, brings together different patterns
- Links isolates over long periods of time with greater certainty

Secondary Impact on Epi/Traceback Evidence

- Reduce some challenges that occur from including unrelated cases in PFGE-defined clusters
  - Example: Diffuse epidemiologic signals become strong when unrelated cases removed
  - Example: Tracebacks do not converge until unrelated cases removed
Challenges for WGS use in Foodborne Illness Outbreak Investigations

Integration
- Transition from PFGE to WGS in outbreak procedures
- Historical comparison

Interpretation
- How close is close enough?
- What does relatedness mean?

Prioritization
- How do we triage clusters for investigation?
- What is our new baseline/background?

Communication
- How do we communicate WGS results to different audiences (firms, lawyers, publicly)?